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Review of the Ph.D. dissertation entitled:
Phylogeography and population genomics of boreal marine macroinvertebrates colonising High
Arctic

by Ph.D. student Hedvig Kriszta Csapó M.Sc.,

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The basis for the preparation of this review is the procedure for awarding the doctoral degree conducted by the Scientific Council of the Institute of Oceanology of the Polish Academy of Science.

The Arctic is under stress. Over the last 40 years, the region has warmed four times faster than the global average, with many effects, among other things, on its ecology, global sea levels, and wider ocean-circulation. However, increasing air temperature is only one of the main factors impacting the Arctic. The reviewed dissertation focusses on another significant factor, ‘Atlantification’, the phenomenon of increasing Atlantic water inflow to the Arctic Ocean, which affects local marine ecosystems. The dissertation combines a review of current scientific knowledge on the biological aspects of ‘Atlantification’ with the original research aimed at investigating the influences of this process on the historical biogeography of selected macroinvertebrates from the most affected area of climatic change, the Svalbard Archipelago.

The Ph.D. dissertation consists of a clearly written bilingual introductory section (Summary in English and Polish) and three thematically related research papers - two of which have already been

published in prestigious, high-impacted scientific journals and one presented as an unpublished manuscript but prepared in a form nearly ready for publication. In the attached statement from the coauthors, the contribution of the Ph.D. candidate is regarded as pivotal at all stages of conducting scientific research and preparation of papers, from the study design (excluding the first paper), through field and laboratory work (if included in the research methodology), statistical data analysis, and finally, the preparation and revision of the manuscript.

The first article presented in the Ph.D. dissertation, entitled “Coming home - Boreal ecosystem claims Atlantic sector of the Arctic”, is written as a paper review and published in the prestigious *Science of The Total Environment*, the very well-impacted journal (IF 10.753 in the year of publication – 2021). The scientific impact of the review is evident from its numerous citations (38 times according to the Scopus database), especially considering the short time since publication. Among the three coauthors, M.Sc. Hedvig Csapó is both the first and corresponding author, which undoubtedly indicates her leading role in the preparation of the publication.

The article is an extensive study of the phenomenon of ‘Atlantification’ in the European Arctic, beginning with an explanation of the term ‘Atlantification’, continuing with a detailed description of its physical manifestations and biological implications. For readers unfamiliar with this topic, the work serves as a valuable source of knowledge, especially since it is based on data from a wide variety of sources (73 publications).

The authors conclude that biological ‘Atlantification’ operates on many different scales and trophic factors. Ecosystem responses to this process include, among other thing, increased primary production, northward shifts of boreal species across different levels of trophic structure, or a gradual decline (or even disappearance) of the local Arctic population. However, in my opinion, the most interesting discussion in the review concerns two concepts:

- i. questioning the general concept of ongoing ‘Atlantification’ driven by anthropogenic influences on climate change, suggesting instead that ‘Atlantification’ is a multiple phenomenon that has recurred in the past, with consequences for the presence of neo-native species among the current colonisers of the Arctic - species that return home every time the climate warms and the ice cover retreats;
- ii. the potential role of floating macroplastic debris, transported to the Arctic by ocean currents, in the dispersal of boreal species.

In the case of the second issue, my question arose: Given the signals in the scientific literature that pathogenic viruses and antibiotic-resistant bacteria also thrive on plastic debris (eg, doi: <https://doi.org/10.1038/d41586-024-03150-6>), is there a danger that such communities of microbes hosted by plastic litter may threaten the Arctic marine ecosystem in light of climate change?

The second part of the doctoral thesis, presented as two original research papers, focusses, in fact, on the empirical testing of an initial hypothesis regarding the multiple ‘Atlantification’ events in the history of climate change, in addition to many other achieved goals.

The first article, entitled “mtDNA data reveal disparate population structure and High Arctic colonization patterns in three intertidal invertebrates with contrasting life history”, was published in well-impacted journal *Frontiers in Marine Science* (IF 2,8 in the year of publication –2023; without citations so far). The Ph.D. candidate holds a key position as the first and corresponding author of this article.

The research examines the population structure, phylogeography, and historical demography of three invertebrate species of boreal origin, found in both the North Atlantic and the Arctic: an amphipod *Gammarus oceanicus*, a gastropod *Littorina saxatilis*, and a barnacle *Semibalanus balanoides*. Without a doubt, the choice of model species for this study is not a matter of chance; it has been considered in-depth by the authors. First, each of them inhabits the intertidal zone, which is more influenced by inflow of Atlantic water due to its unique position at the interface of land and sea. Second, these species differ in their reproductive strategies and consequently in their dispersal mobility. Third, the species' wide distribution allows for a broad sampling area, including the first investigation of the Svalbard population of these species. The research utilized a large number of COI sequences - a mitochondrial DNA marker widely used in animal biodiversity studies, particularly in the DNA barcoding approach. The dataset includes (711) newly obtained sequences as well as (1 492) reference sequences deposited in public databases. Using well-chosen and appropriate bioinformatic methods sufficient to achieve the established goals enabled the drawing of reliable results. The main conclusions of the study are:

- i. colonisation of the Arctic by each of the three boreal species originated from the eastern Atlantic;
- ii. the expansion probably occurred during an early Holocene warm cycle, consequently excluding the potential influence of the ongoing ‘Atlantification’ on this process;
- iii. a higher potential for dispersion (as seen in *S. balanoides*) correlates with a weak population structure, while low mobility (in *G. oceanicus* and *L. saxatilis*) leads to a well-defined population structure.

I enjoyed reading the article and appreciate the tremendous amount of work and the professional preparation and presentation of the results. I have a question:

How does the use of estimated molecular clock rates from studies like Loeza-Quintana et al. (2019) influence the interpretation of evolutionary timelines for Arctic studied invertebrates? Are there available data on the fossil record for any marine arthropods, and if not, how does their absence affect the reliability of biogeography-based calibrations in these studies?

The second, unpublished manuscript, entitled “Distinct population structure and demographic history of the northern acorn barnacle argue against its spread in the High Arctic as a result of recent Atlantification,” is an interesting continuation of the research, this time using the low coverage whole genome sequencing (LC-WGS) method - a valuable tool for population genomics and biogeography analyses. The research examines the population structure of *Semibalanus balanoides*, as well as its origin and timing of colonisation in the Svalbard archipelago. Two competing hypotheses are evaluated: the contemporary Atlantification of the Arctic versus expansion that occurred at the end of the last glacial maximum (LGM).

First, the results of the demographic reconstruction indicate that the divergence of the Svalbard population from the European population occurred during the Holocene Thermal Optimum. Second, genetic structure analyses revealed a strong geography-influenced pattern, although *Semibalanus balanoides* has a pelagic larva in its life cycle, suggesting a high potential for dispersal. This is surprising, given that earlier studies based on a single mitochondrial marker suggested homogeneous rather panmictic populations of this species in Svalbard. This just shows that each result should be approached with moderate optimism, and results based on the sensitivity of single genetic markers may not be sufficient in studies of population genetic structure or demographic history. Therefore, it is worth appreciating the intuition of the authors to validate the initial results.

My question is: Taking into account the results of both studies, how do you view the balance between using a single marker and whole genome data in terms of accuracy and reliability in your research? Do you see the limitations that come with inference based on whole genome data?

In conclusion, I fully agree with the authors' statements from the discussion of the first research article of the Ph.D. dissertation by M.Sc Hedvig Csapó, that the study „provides fundamental information on the genetic composition, diversity, and population structure of the analysed species in the North Atlantic and Arctic regions." Among the many results obtained, it is particularly worth emphasising that the research provides new and original information on the phenomenon of ‘Atlantification’. In particular, the innovative findings suggest that “Atlantification” is not merely a contemporary process driven by human-induced climate change, but rather involves a longer timescale shaped by recurring glacial and interglacial changes.

All the research papers in the Ph.D. dissertation by M.Sc. Hedvig Csapó has been carefully designed, using appropriate research methods and statistical tools to support the discussion and meet the goals. The Ph.D. candidate not only skilfully introduces the topic of her research (a paper review), but also clearly formulates research hypotheses in each of the presented research papers. The results are clearly presented, and M.Sc. Hedvig Csapó interprets them skilfully, drawing from the latest

literature in her research field. She has demonstrated the ability to approach her findings critically and draw effective conclusions.

The reading of the dissertation leaves no doubt that the M.Sc. Hedvig Csapó has gained extensive knowledge and skills in conducting independent research using a wide range of molecular techniques, including modern ones. Therefore, it is not surprising that two of her published papers have also been recognised by the Editorial Board and reviewers of two prestigious journals.

In the case of the unpublished work, I see a few minor shortcomings regarding the editing and formatting of the text, the lack of references to tables and figures in the Results section, and the absence of full forms for abbreviations in the text. Attention should be paid to these issues when preparing the work for publication. Additionally, the Polish version of the Summary has some editorial shortcomings compared to the English version. However, this does not affect the exceptionally high quality of the presented doctoral work.

Considering the scientific value of the presented Ph.D. dissertation, I declare that it meets all the requirements for a Ph.D. thesis as specified in Article 187, paragraphs 1-4 of the Act of July 20, 2018, the Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended). I have requested the Scientific Council of the Institute of Oceanology, Polish Academy of Sciences, to admit M.Sc. Hedvig Kriszta Csapó to the further stages of the dissertation process. At the same time, considering the high scientific value of the presented dissertation and the original solution to the scientific problem, I request that the Ph.D. thesis be awarded a distinction.

Hysocika
